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In vitro Soluble CD30 Levels in Patients with Chronic Stable Coronary Artery Disease

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ABSTRACT

The CD30 antigen seems to play a costimulatory role in maintaining the physiological balance between T-helper (Th)1/Th2 immune responses. In this study, plasma and *in vitro* soluble CD30 (sCD30) secretion was investigated in patients with coronary artery disease (CAD) as a plausible marker of dysregulated immune response.

Twenty one patients with angiographically confirmed CAD and 31 healthy controls took part in this study. The levels of the activation marker sCD30 were determined in plasma and phytohaemagglutinin (PHA)-stimulated and unstimulated peripheral blood mononuclear cell cultures by ELISA.

Plasma sCD30 levels did not differ significantly between the patients and controls. However, spontaneous sCD30 secretion was significantly lower in patients with CAD compared to controls ($p < 0.001$). The soluble CD30 levels were significantly increased in the supernatant of PHA-stimulated PBMCs compared to unstimulated cultures in both groups of patients and controls ($p < 0.001$). PHA-stimulated sCD30 secretion was found to be lower in patients compared to controls; however, the difference was not statistically significant.

Plasma sCD30 levels were not statistically different in patients with chronic stable CAD, a well-known Th1-mediated disease, compared to controls; whereas decreased spontaneous and PHA-stimulated sCD30 secretion in patients with CAD might indicate the progressive shift towards a Th1 immune response.

Keywords: Coronary artery disease; Soluble CD30; T-helper

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INTRODUCTION

Atherosclerosis is a chronic inflammatory disease of the arterial wall characterized by progressive lipid

accumulation, inflammatory cell infiltrates, cell death, and fibrosis.[1] The immune system, encompassing both innate and adaptive immunity, has been implicated in the atherogenic process.[2,3] Despite the fact that the precise role of various T-cell subsets in atherogenesis is far from clear, the proatherogenic role of T-helper type 1 (Th1) cells is well established, promoting inflammation and matrix degradation.[4]

CD30 is a membrane glycoprotein of the tumor necrosis factor receptor (TNFR) superfamily,[5] originally identified as a cell surface antigen on Reed-Sternberg cells of Hodgkin lymphoma.[6] In normal conditions, CD30 expression depends on cell activation and proliferation and is largely restricted to activated T, B, and natural killer cells.[7] Previous studies have yielded conflicting results concerning the preferential expression of CD30 on lymphocytes expressing a Th2 phenotype.[8-11] One explanation would be that CD30 has been found to be transiently expressed on normal activated T cells, whereas its expression persists on T cells of Th2 profile.[7,12] Far more than being a mere marker, CD30 exerts pleiotropic biological functions,[8,12] which, of note, is its costimulatory role in maintaining the physiological balance between Th1/Th2 immune responses.[13-15]

A soluble form of CD30 (sCD30) has been found to be closely related to cell CD30 expression.[16,17] Increased sCD30 levels have been reported in a variety of immunopathological disorders;[12,18,19] however, it is still a matter of debate whether abnormal increases or decreases in sCD30 levels are associated with impaired regulation.[14,15] Indeed, to our best of knowledge, this is the first time that this biomarker is assessed in coronary artery disease (CAD). The purpose of the present study was to investigate plasma and *in vitro* secretion of sCD30 in phytohaemagglutinin (PHA)-stimulated and unstimulated peripheral blood mononuclear cell (PBMC) cultures in patients with chronic stable CAD, as a plausible marker of dysregulated immune response, in comparison with healthy controls.

MATERIALS AND METHODS

Participants

In the present study, a total of 52 participants who underwent elective coronary angiography at the Catheterization Laboratory of Imam Khomeini Hospital Complex, Tehran University of Medical Sciences for the evaluation of stable CAD based on clinical indication were enrolled. Twenty one patients with significant CAD, defined as ≥ 50% diameter stenosis in at least one of the major coronary arteries, and 31 subjects with no CAD took part in this study. Subjects with active infections, autoimmune diseases, malignancies, and recent myocardial infarction were excluded. Written informed consent was obtained from all participants prior to blood sampling. This study was approved by the Ethics Committee of Tehran University of Medical Sciences and Health Services.

Cell Culture

The heparinized blood samples were centrifuged and the plasma was collected and stored at -80ºC until assayed. PBMCs were isolated by the Ficoll-Hypaque gradient centrifugation method. The remaining whole blood was diluted 1:2 with sterile phosphate-buffered saline PH=7.4 and layered onto half the volume of Ficoll-Histoprep (BAG Health Care GmbH, Germany). The sample was centrifuged and the interface layer containing the mononuclear cells was collected. Cell viability and cell counts were assessed by Trypan blue exclusion method. Mononuclear cells were resuspended in culture medium (RPMI-1640 supplemented with 10% heat inactivated fetal calf serum; Gibco, Invitrogen, UK), diluted to 1×10^6 cells per mL, and plated at a density of 1.5×10^5 cells per well in 96-well flat-bottomed microtiter plates. Cells were cultured for 66 hours in the presence of 10 µg/mL PHA (Sigma, USA) or medium alone. Culture supernatants were harvested and frozen at -80ºC for sCD30 assay.

sCD30 Assay

sCD30 concentrations were measured in plasma and supernatants of PBMC using commercially available Enzyme-Linked Immunosorbent Assay (ELISA; Bender MedSystems, Vienna, Austria). The optical density was determined at 450 nm using micro ELISA plate reader. sCD30 concentration was read from the standard curve generated using recombinant human sCD30 provided with the assay kit. The results obtained were expressed as ng/mL.

Statistical Analyses

The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Parametric and nonparametric variables were presented as mean ± SD and median (range), respectively. Mann-Whitney U test and

Wilcoxon signed ranks test were used to compare variables between groups. A p value < 0.05 was considered statistically significant.

RESULTS

Twenty one patients with angiographically confirmed CAD aged 40-69 (51.62 ± 9.13) years and 31 healthy controls with no coronary artery involvement aged 42-66 (49.90 ± 7.04) years took part in this study (p = 0.161). Nineteen out of 21 (90.47%) patients and 28 out of 31 (90.32%) controls were male.

Plasma sCD30 levels did not differ significantly between the patients and controls (p = 0.225); whereas, *in vitro* spontaneous sCD30 secretion was significantly lower in patients with CAD (median: 0.00 ng/mL, range: 0.00-2.01 ng/mL) compared to controls (median: 0.00 ng/mL, range: 0.00-8.90 ng/mL) (p < 0.001) (Figure 1).

Following PHA stimulation, the levels of sCD30 secretion were significantly increased in both groups of patients (p < 0.001) and controls (p < 0.001). Likewise, PHA-stimulated sCD30 secretion was found to be lower in patients with CAD (median: 7.59 ng/mL, range: 0.00-22.60 ng/mL) in comparison with controls (median: 8.64 ng/mL, range: 0.00-34.04 ng/mL), but this difference was not statistically significant (p = 0.332).

DISCUSSION

CD30 has been found to be an important costimulatory molecule for maintaining the physiological balance between Th1/Th2 immune responses.[13-15] Despite early reports of normal serum sCD30 levels in Th1-driven diseases,[20,21] increased levels of sCD30 and/or CD30 expression have been demonstrated in subsequent studies of Th1-polarized immune responses, including tuberculosis,[22] Wegner's granulomatosis,[23] Hashimoto's thyroiditis,[24] Graves' disease,[24] Graves' ophthalmopathy,[25] primary biliary cirrhosis,[26] Sjogren's syndrome,[27,28] and rheumatoid arthritis.[29,30]

It has been postulated that $CD30^+$ T cells play a counter-regulatory role in rheumatoid arthritis and, by extension, in other Th1-mediated conditions, with increased circulating sCD30 levels reflecting such cell activity.[31]

This may have been partly due to increased production of interleukin (IL)-4 and IL-10 by $CD30^+$ T cells.[30] Indeed, the production of Th2-type cytokines counteracts the deleterious effects of proinflammatory Th1-type cytokines in an attempt to resolve the disease process.[32-34]

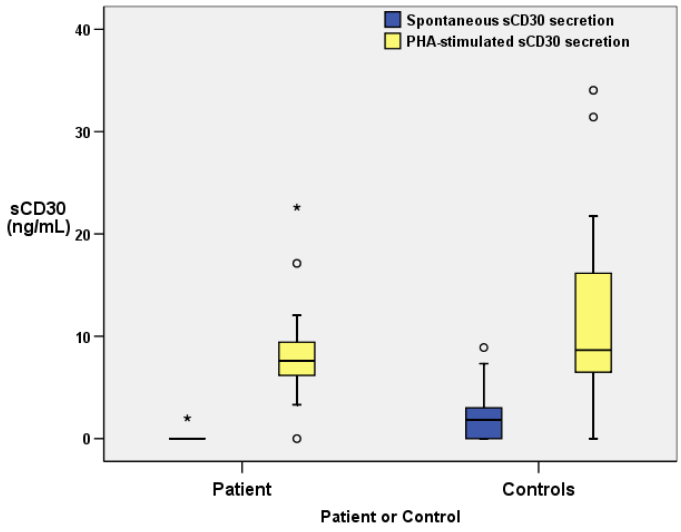

Figure 1. Spontanous and PHA-stimulated sCD30 secretion (ng/mL) in patients with CAD (n=21) and controls (n=31). Boxes represent values between the 25th and 75th percentiles. The horizontal lines correspond to the median, minimum and maximum. A p value < 0.05 was considered statistically significant.

The serum sCD30 levels were inversely correlated with the inflammatory marker C-reactive protein in early rheumatoid arthritis and proved to be of prognostic value in predicting clinical response to second-line therapy,[35] further supporting the above hypothesis. In the present study, plasma sCD30 levels were not statistically different in patients with chronic stable CAD, a well-known Th1-mediated disease,[4] compared to controls. However, *in vitro* sCD30 secretion was significantly lower in patients than controls, further complicating our understanding of its pleiotropic regulatory roles within the immune system. To our best of knowledge, the significance of sCD30 levels in patients with CAD has not been reported previously.

CD30 signaling has been demonstrated to prevent extensive expansion of autoreactive $CD8^+$ T cells upon secondary antigenic encounters in peripheral parenchymal tissues, such as pancreatic islets, thus protecting against autoimmunity.[36] Furthermore, antigen-induced $CD4^+CD25^+$ regulatory T (Treg) cells have been found to suppress allograft rejection through enhanced memory $CD8^+$ T cell apoptosis, a finding which was largely dependent on the presence of CD30 on Treg cells and intact CD30-CD30L interaction. [37] Taken together, these studies illustrated a novel regulatory role for CD30 signaling in autoimmune diseases, i.e. CD30-mediated apoptosis of memory $CD8^+$ T cells, to eliminate self-reactive immune effector cells.[12] Current evidence supports an autoimmune mechanism in the pathogenesis of atherosclerosis, with oxidized low-density lipoproteins, heat shock proteins, and β2 glycoprotein I being identified as the culprit autoantigens in the development and progression of the disease.[38,39] Thus, decreased $CD30^+$ T cell populations and/or CD30 expression may potentially enhance atherogenesis through decreased elimination of autoreactive memory $CD8^+$ T cells. Nevertheless, the precise role of $CD8^+$ T cells in atherogenesis and plaque destabilization is yet to be elucidated.[4]

It is still unclear whether the progressive shift towards a Th1 immune response influences CD30 expression[30] or lower than normal $CD30^+$ T cell activity results in Th1 predominance.[40] In the latter case, manipulation of CD30-CD30L interaction will be of therapeutic value in Th1-mediated conditions. Furthermore, lower sCD30 levels in patients with angiographically confirmed CAD might represent an inherited susceptibility to the development of the disease, such as yet unidentified gene polymorphisms associated with autoimmunity.[12]

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